Determining Factors of Breast Cancer Survival

Daehee Kang, Ji-Yeob Choi
Molecular Genomic Epidemiology Laboratory (MGEL)
Seoul National University

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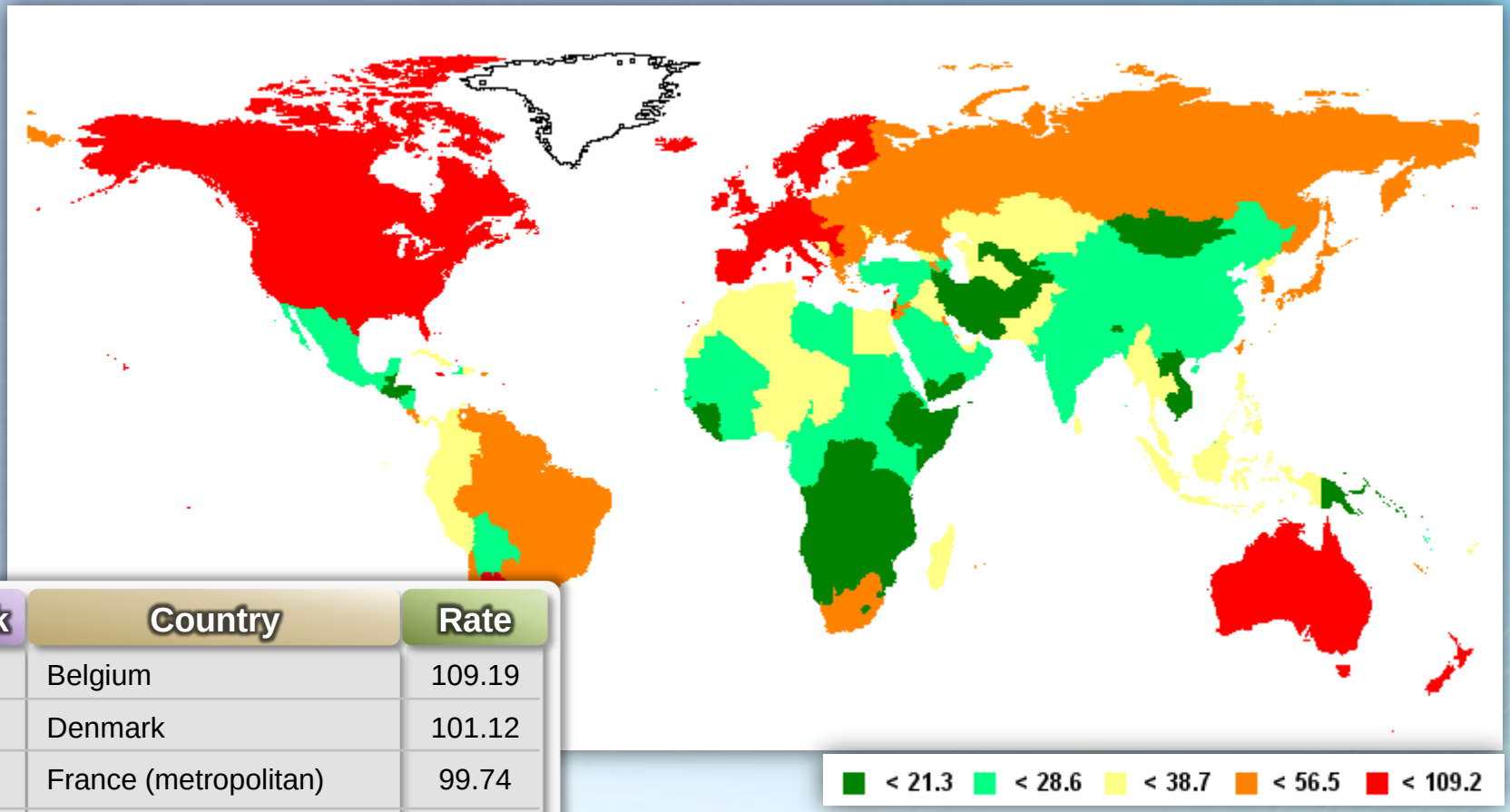
Molecular & Genomic Epidemiology Lab

Outline

- Epidemiology of breast cancer subtypes
- Factors related to breast cancer prognosis
- Biomarkers for breast cancer survival
  - GWAS, methylation, and serum protein markers
- Summary and future direction

Estimated Age-Standardized Incidence Rates



Rank	Country	Rate
1	Belgium	109.19
2	Denmark	101.12
3	France (metropolitan)	99.74
…	…	…
65	Japan	42.71
75	Korea, Republic of	38.89
145	China	21.64

Per 100,000 Breast, all ages; GLOBOCAN 2008

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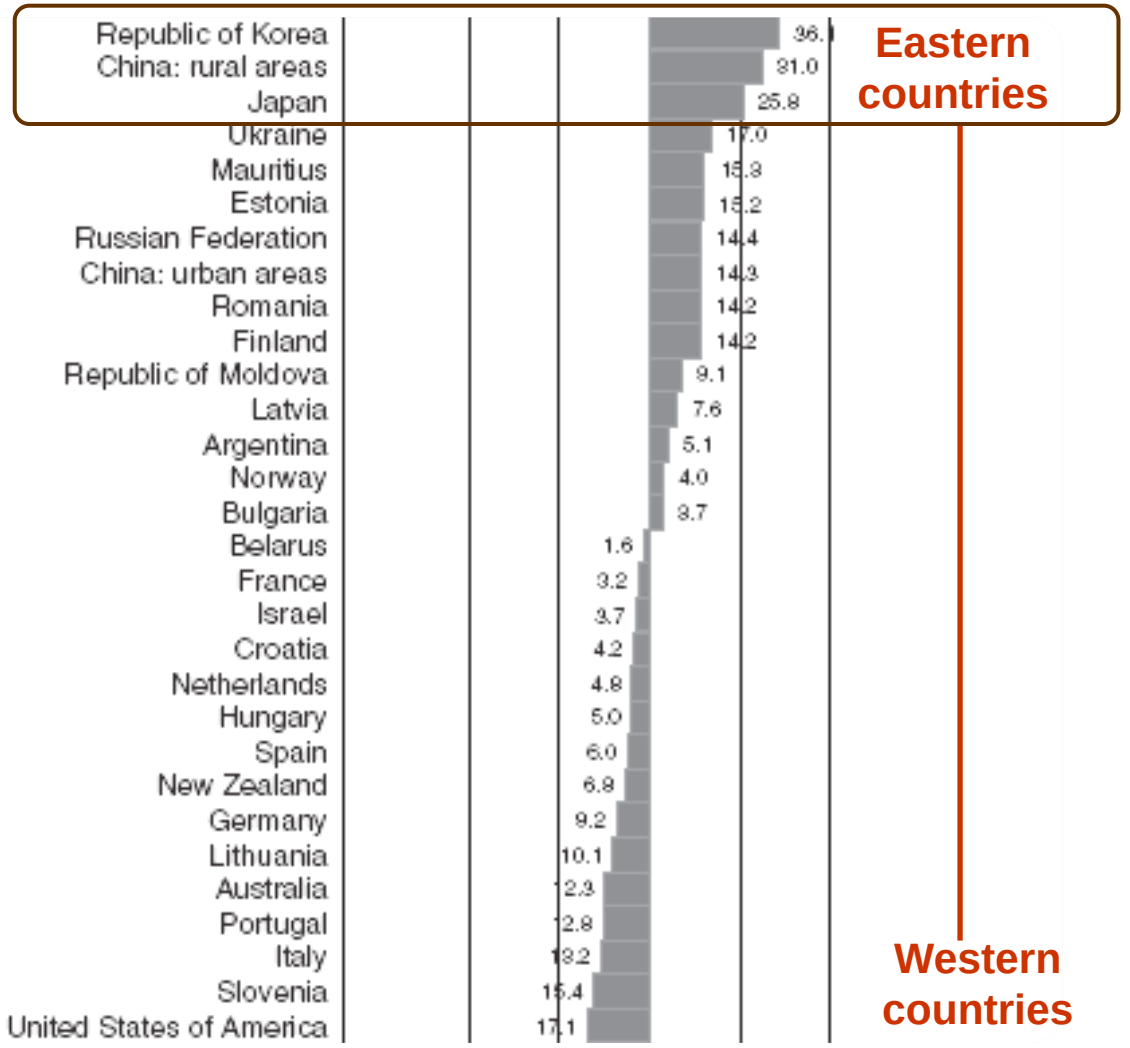
Change in Breast Cancer Mortality (1985-1995)

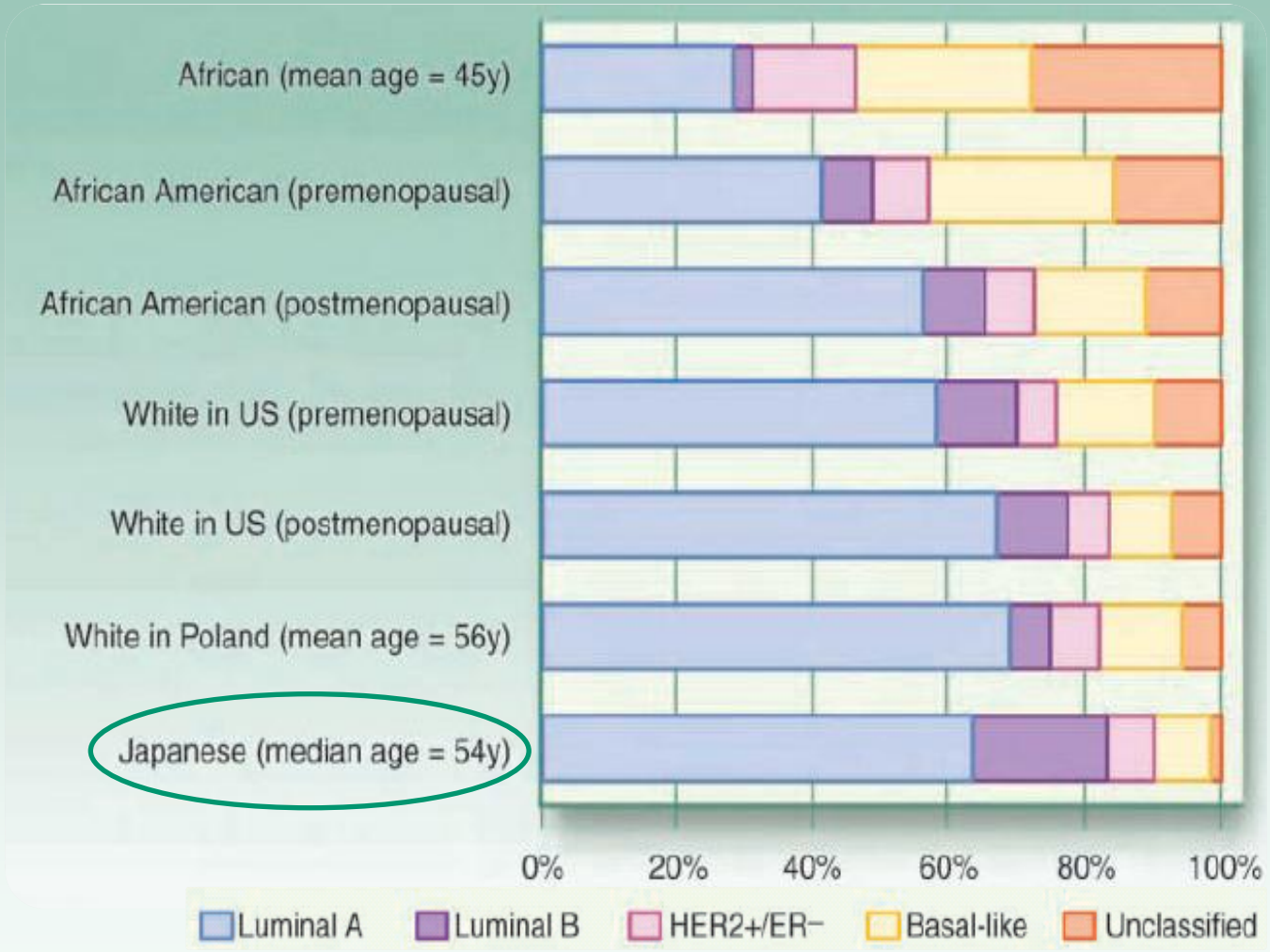
MRs in low incidence area showed marked increase



MRs in Western countries decreased
← earlier detection & improved treatment

Bray et al, 2004, BCR

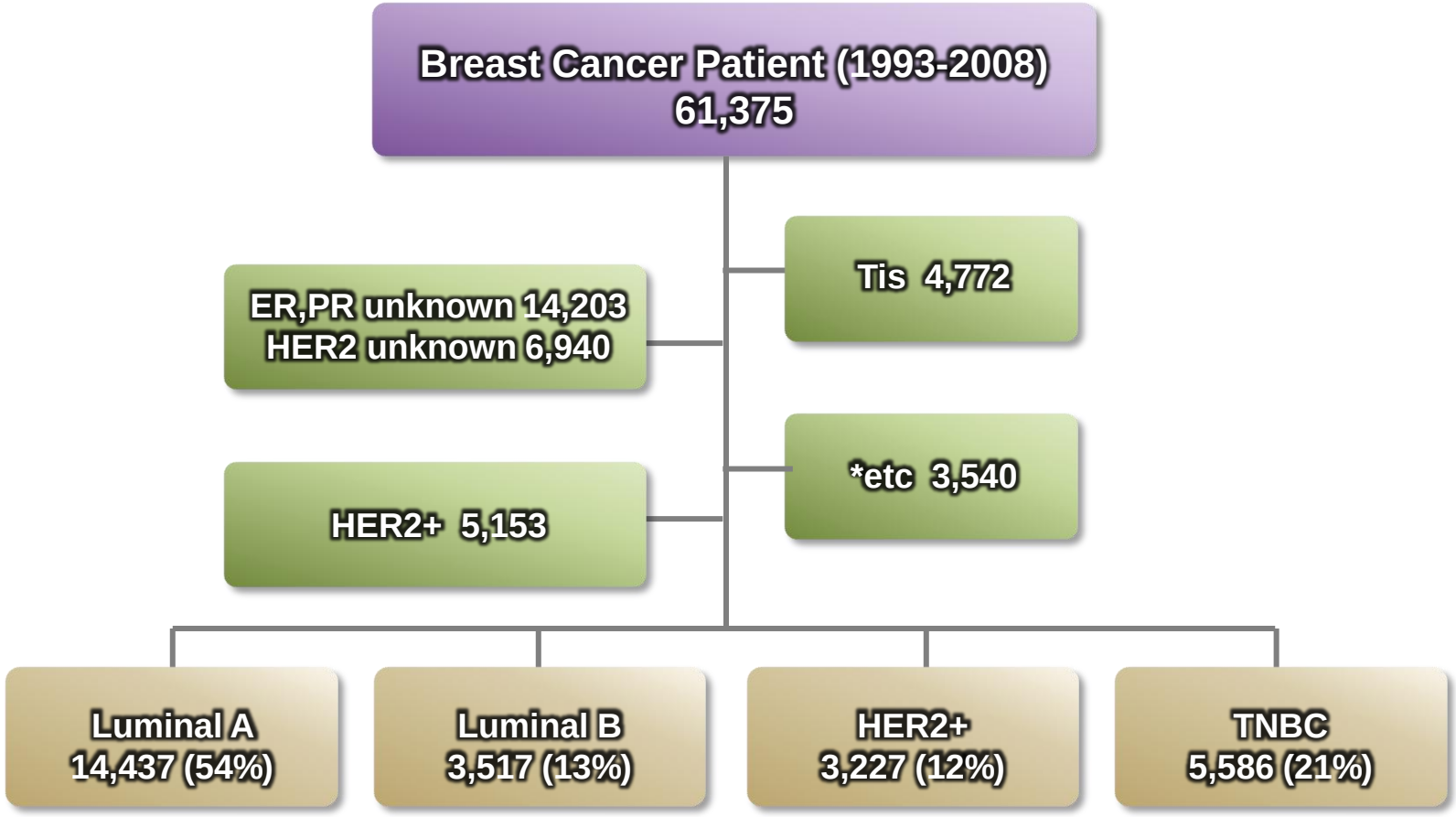
Distribution of Breast Cancer Subtypes Across Different Populations



Olopade et al, 2008, CCR

Subtypes of Breast Cancer in Korea

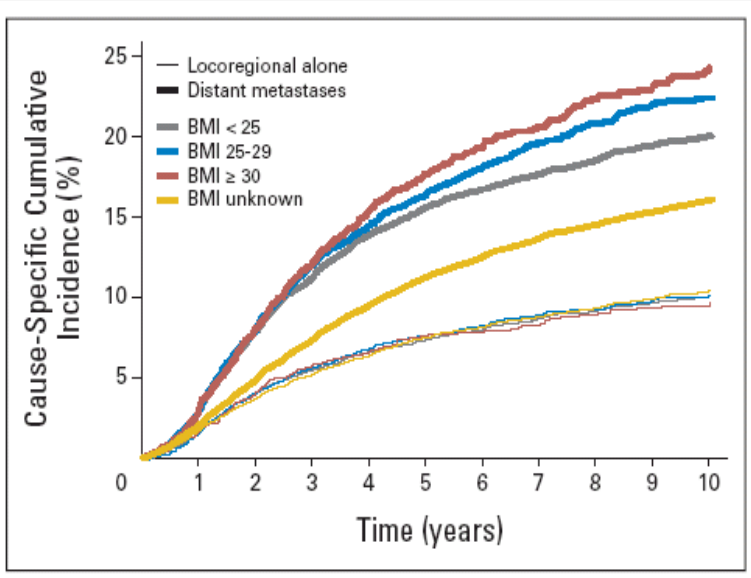
using Korean Breast Cancer Society Registration data



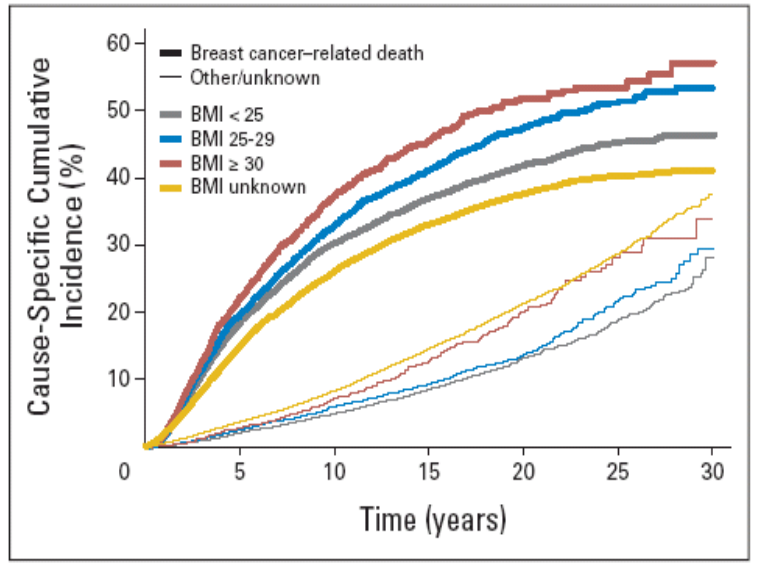
Lee et al, 2010, BCRT

Obesity and Prognosis of Early-Stage Breast Cancer (Danish, n= 53,816)

Cumulative incidence of first events



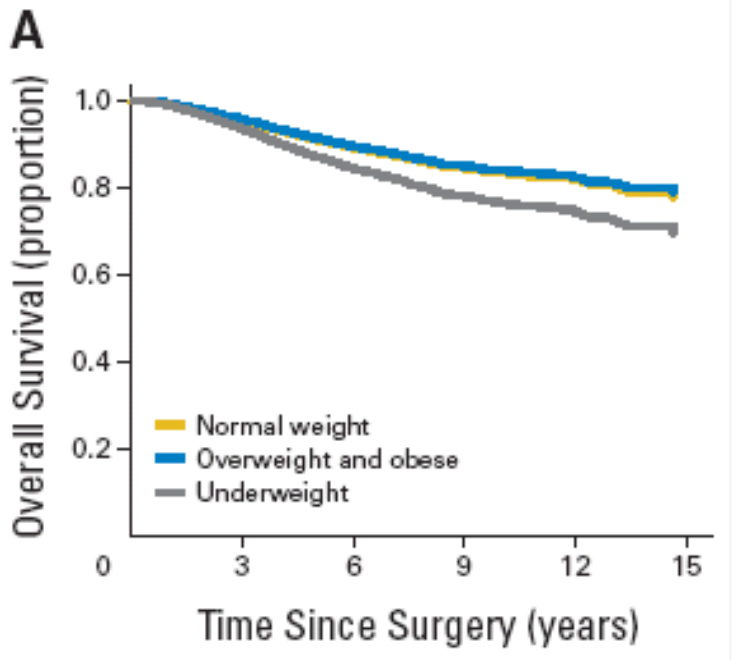
Risk of death



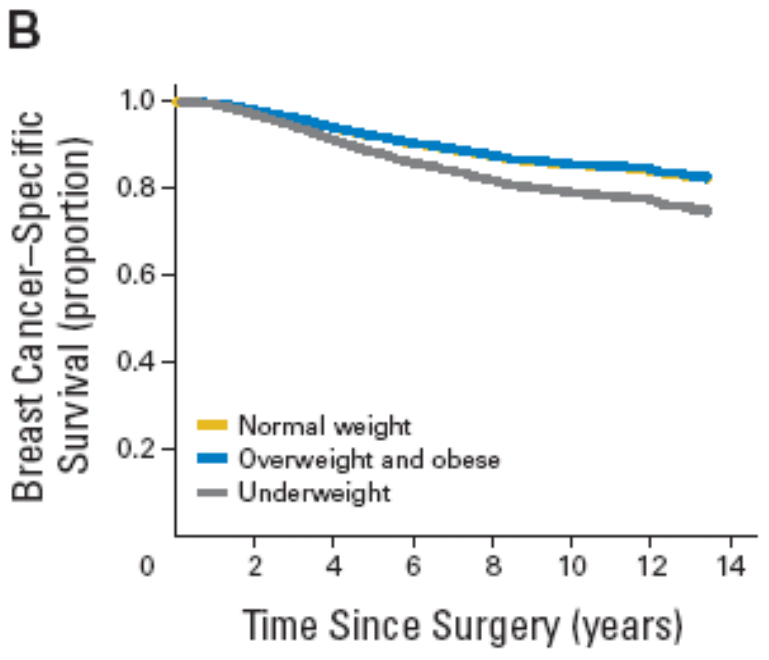
Ewertz et al, 2010, JCO

Underweight and Breast Cancer Recurrence and Death in Korean (n= 24,698)

Overall survival



BrCa specific survival



Underweight <18.5, Normal 18.5-24.9, Overweight and obese > 25.0+

Moon et al, 2009, JCO

Seoul Breast Cancer Study(SeBCS)

- a multicenter hospital based case-control study
  - since 1995
  - SNUH, AMC

  - 4,000 cases and 2000 controls (1995-2007)
  - Interview, biological specimen, medical record

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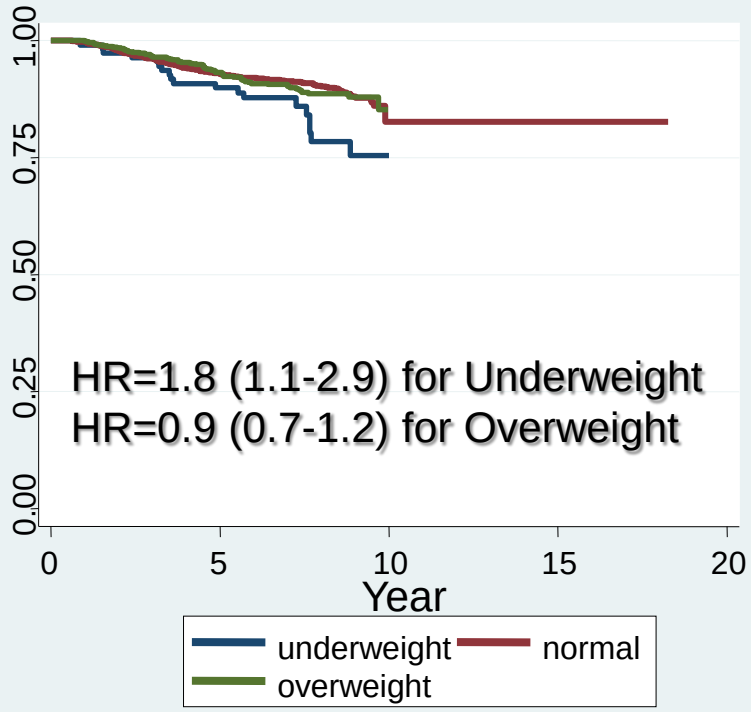
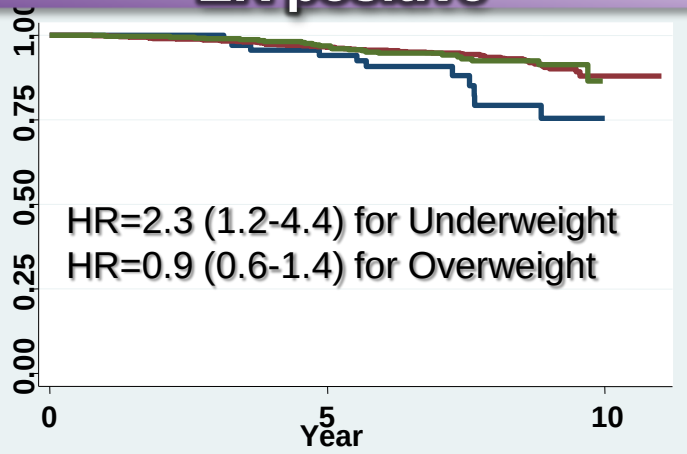
Underweight and Breast Cancer Survival in SeBCS

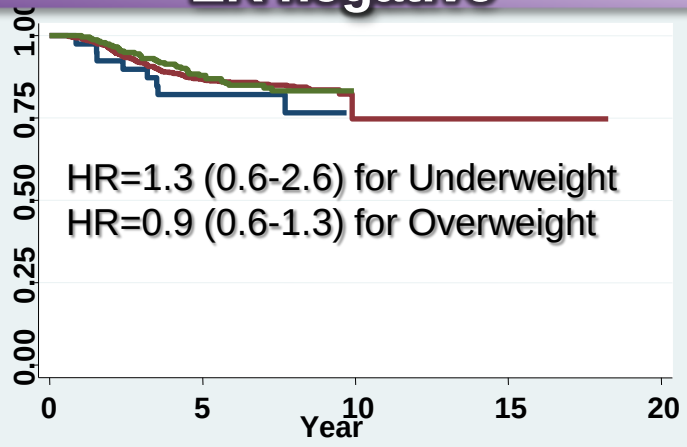
All participants



ER positive

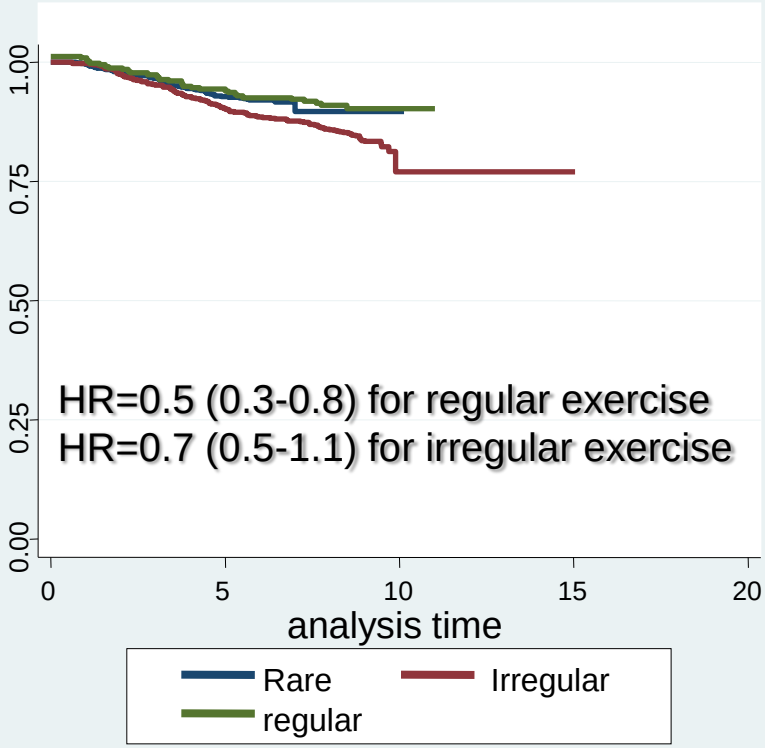


ER negative

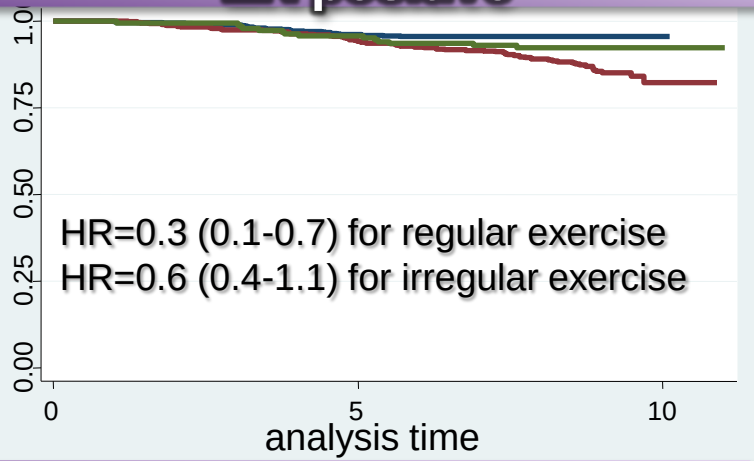


Physical Activity and Breast Cancer Survival in SeBCS

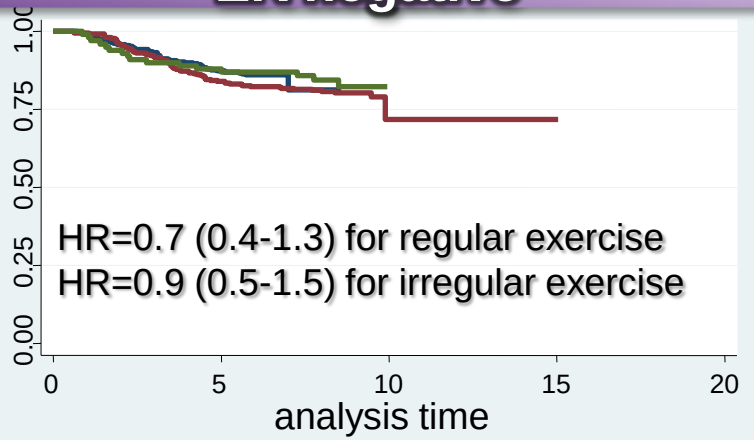
All participants



ER positive



ER negative



SNPs Related to Breast Cancer Prognosis

Sample size	Candidate genes (1-2 genes)	Genes in the same pathway
<100	*XRCC1*	
100-500	*ST14/SPINT1* *SOD2* *IL-2* *NPAS2*	ADMET Folate metabolism IL related
500-1000	*PIK3CA* *CYP2C8/9*	*VEGF/IL/TGFb* Telomerases Chromosomal instability
1000+	*CYP19A1* *C1QA* *P53/MDM2* *LEP/LEPR (w/obesity)* *SIPA1, RRP1B* *SIPA1* *MMP7* *hCDC4/cyclinE*	Estrogen metabolism *TIMP2/3*
5000+		Prostaglandin related

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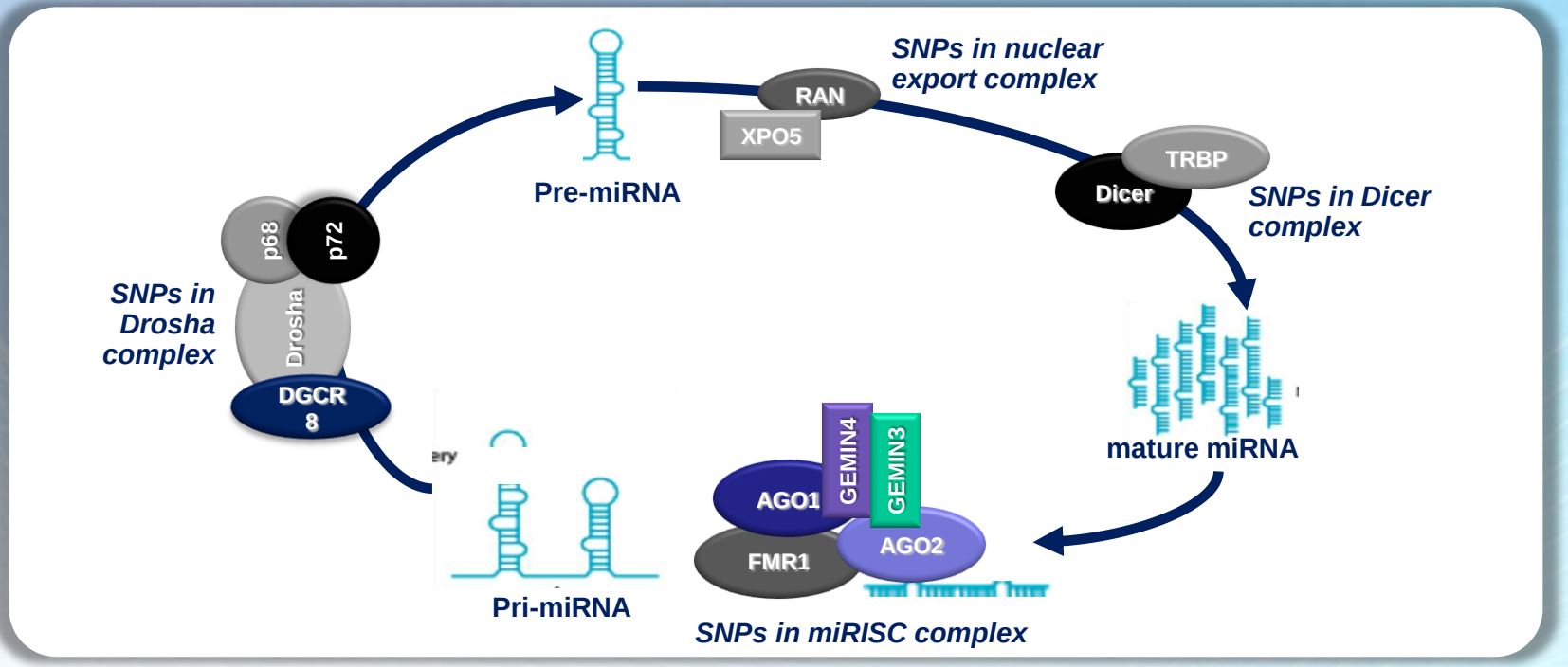
microRNA Biogenesis Pathway Genes

- **Total 41 haplotype tagging SNPs in 14 miRNA biogenesis pathway genes**
  - 35 SNPs in 12 miRNA-processing machinery genes (*AGO1, AGO2, DICER1, DGCR8, DROSHA, FMR1, GEMIN3, GEMIN4, HIWI, RAN, TARBP2,* and *XPO5)*
  - 6 SNPs in 2 genes to regulate estrogen-mediated miRNA processing (*p68* and *p72*)



SNPs in microRNA Biogenesis Pathway genes and survival in SeBCS (n=488)

Gene	SNP	DFS, HRa (95% CI)	OS, HRa (95% CI)
AGO2	rs2292779	1.40 (1.05- 1.87)	2.26(1.18- 4.35)
	rs11786030	2.52 (1.37- 4.65)	2.63(1.15- 6.02)
DGCR8	rs9605062	0.61 (0.37- 1.00)	0.59(0.28- 1.22)
	rs9606250	0.23 (0.06- 0.93)	-
DROSHA	rs874332	1.10 (0.83- 1.45)	1.98(1.07- 3.64)
HIWI	rs4759659	0.48 (0.28- 0.82)	0.59(0.29- 1.23)

NOTE Disease free survival event included locoregional recurrence (n=76), 2nd primary cancer (n=11), and death (n=3) from any cause.

[a] Adjusted for age, TNM stage, and nuclear stage.

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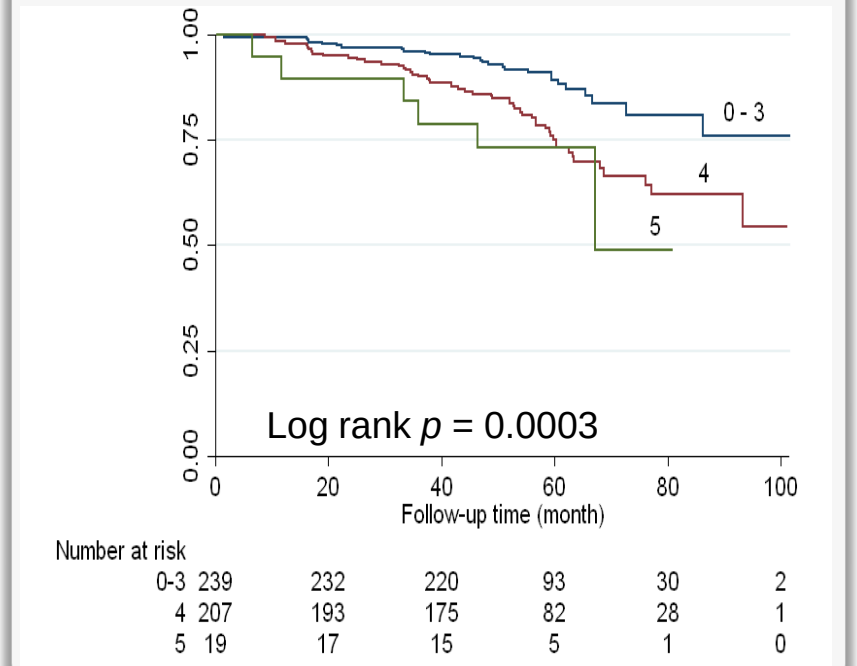
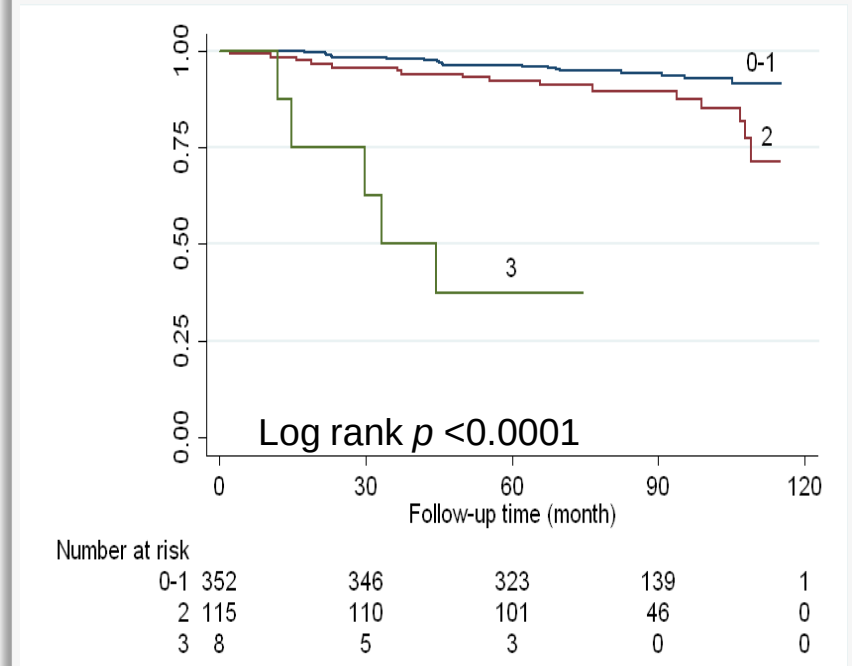
Combined Effect of Unfavorable Genotypes and Breast Cancer Survival in SeBCS

DFS



OS



Unfavorable genotypes defined based on single SNP analysis and their best fitting model : rs11786030 (AG+GG), rs2292779 (GG), rs4759659 (GG), rs9605062 (TT), and rs9606250 (AA+AT) for DFS and rs874332 (CC), rs2292779 (GG), and rs11786030 (AG+GG) for OS

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Global Methylation and Breast Cancer Subtypes in SeBCS

1st stage

Genomewide scan

- Design: 12 ER+/PR+ vs. 12 ER-/PR-
- Method: Illumina Infinium methylation assay (27,578 CpG sites)

2nd stage

Validation study

- Design : 31 ER+/PR+ vs. 39 ER-/PR-
- Method:
  - MSP (PER1, and MANEAL)
  - pyrosequencing (FAM124, T6GALNAC1, NAV1)

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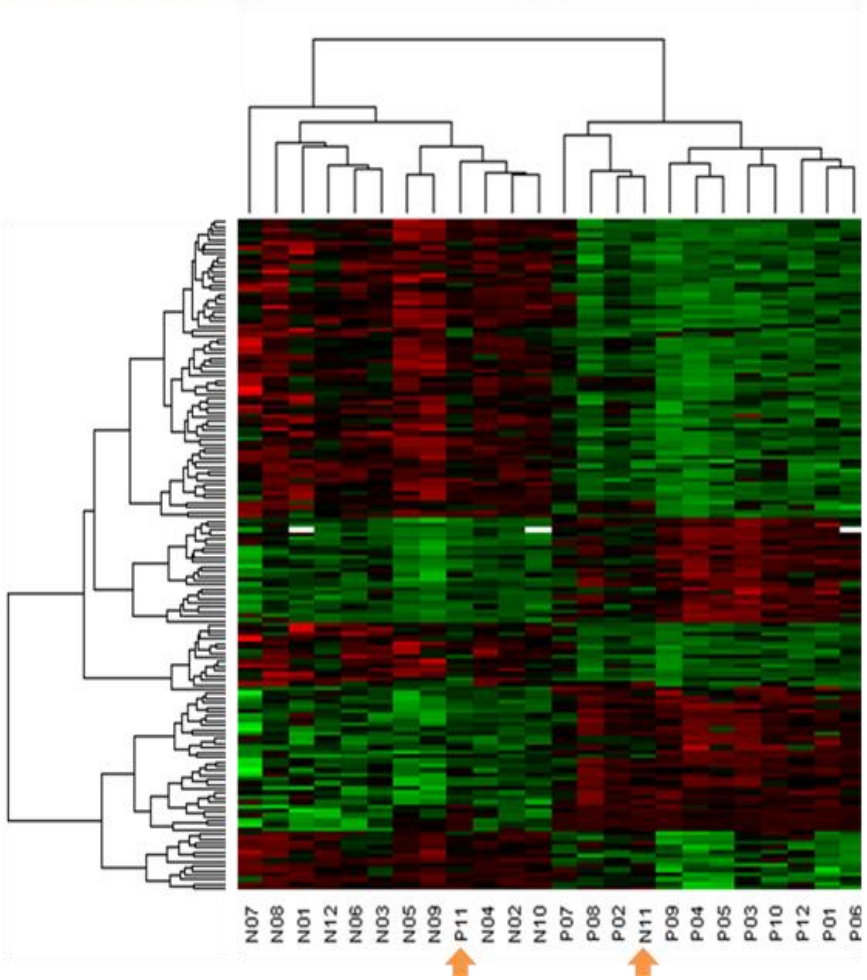
Cluster Analysis of Methylation Level between ER+/PR+ and ER-/PR- Breast Cancer

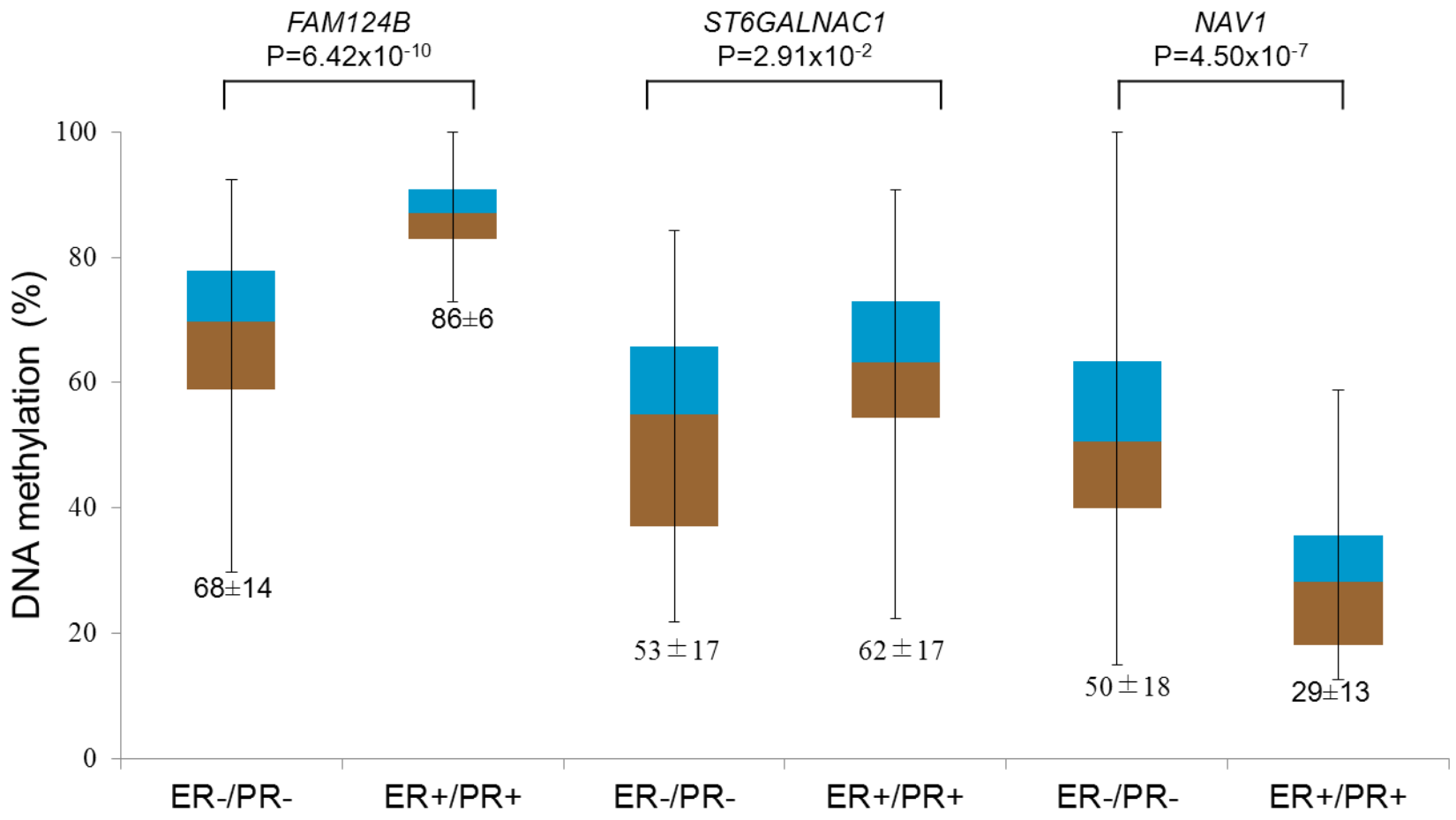


ALDH1A3	CENPH	HAMP	MSX1	SH3TC1
AMT	CHRD	HAVCR2	MYF5	SLPI
ANK1	COL17A1	HEXIM1	MYF6	SORBS3
ANXA9	CPA3	HGFAC	NAV1	ST6GALNAC1
APXL2	CST9L	HSD3B1	NCF1	ST6GALNAC4
ASB10	CYB5R2	HSPA12B	P2RX1	SYN3
ASB16	CYP2A13	HSU79303	P2RXL1	TBX19
BGLAP	CYP2A7	HTR3D	P518	TBX4
BOK	DAPK1	IL8RA	PER1	TCP10
BSCL2	DNALI1	ISL2	PLAT	TLR9
BZW1	ECOP	ITGAM	PLCB2	TMC6
C10orf39	EPN3	JMJD4	PLXNB1	TMEM71
C14orf93	EPOR	KCNJ3	POLR2D	TNFAIP8
C16orf50	FAM77C	KCNJ9	POLR2I	TOP1MT
C18orf16	FCGR2A	KSR1	PPP1R7	TPSG1
C1QL1	FCGR2B	KYNU	PRKAR2B	TRAK1
C20orf135	FGD2	LOC348174	PRP2	TREML2
CARS	FGF12	LTBP3	PSMD6	TRIM22
CBLC	FLJ11017	MAG	PTPN18	UCK1
CBX7	FLJ14346	MANEAL	PTPN22	VWCE
CCL3L1	FLJ22746	MGC39715	RASIP1	WFDC10B
CD22	FLJ31568	MGC52423	RHOC	ZBTB16
CD28	FLJ43276	MLC1	SBEM	ZNF491
CD320	FLJ43806	MLZE	SCNM1	
CD79A	FLJ44112	MRPL30	SEMA3B	
CDK2	FLJ90575	MS4A12	SERPINA12	
CDKN2B	GNAS	MSR1	SH2D2A	

(ER+/PR+: P01~P12, ER-/PR-: N01~N12, 148 methylation sites, $p<0.001$)

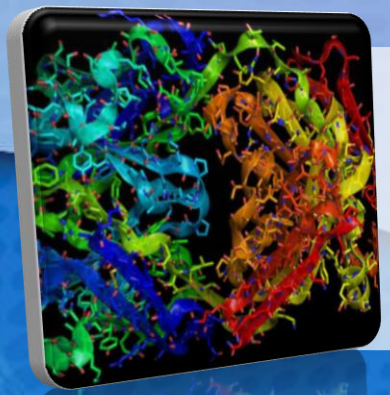
Li and Kang, 2010, HMG

Results of Replication Study using Pyrosequencing in SeBCS



Li and Kang, 2010, HMG

Serum Biomarkers, Under Research

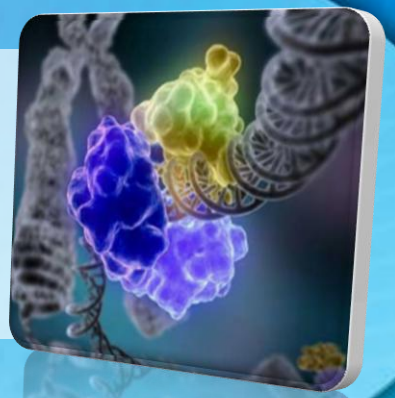


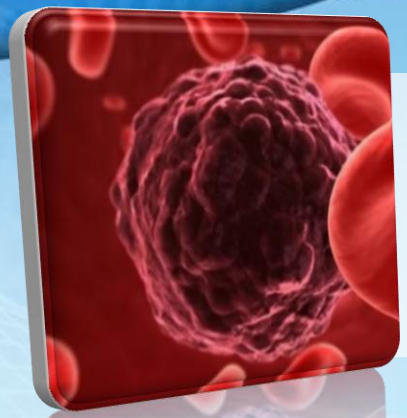
Proteins

Autoantibodies
Cytokines

Cell free DNA-RNA

DNA
Small RNA





Circulating tumor cells

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Serum Protein, Under Research

Biomarkers	Outcomes
Members of the MUC family (MUC2,3,4,5,6)	Prognosis
Fragment of Cytokeratin 19, 18	Diagnosis
Circulating HER2 status	Recurrence Response to therapy
Mammoglobin, kallikrein 14, osteopontin, mutant p53	Diagnosis
Plasma prolactin	Diagnosis
IGF-1 & IGFBP-3	Diagnosis
bFGF &VEGF	Diagnosis
VEGF, Tie-2, angiopoietin-1	Prognosis Response to therapy
TIMP-1	Survival in metastatic BC
Pro-MMP	Aggressive behavior
Preoperative E-selectin	Advanced stage

Serum Proteins Explored in SeBCS

- Lipotoxicity; adiponectin, leptin, hsCRP, HGF, resistin, RBP, lipocalin-2, adipsin
- Angiogenesis; b-FGF, VEGF, MMP2/MMP9, TIMP2
- Oxidative Stress; hsp70/hsp70b
- One-carbon metabolism related to methylation; folic acid, homocystein

Possible Mechanisms of Lipocalin-2/MMP9 in Promoting Mammary Tumorigenesis and Metastasis

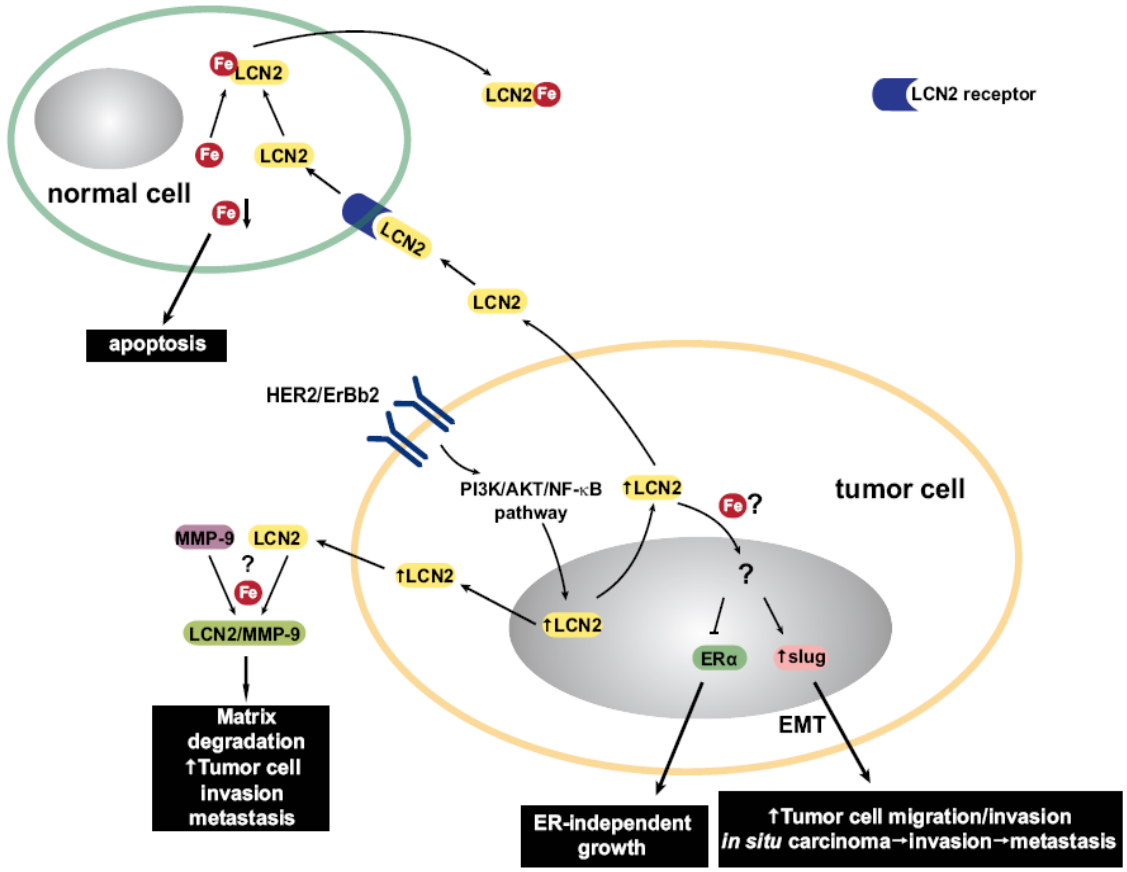


Fig. 1. Possible mechanisms of LCN2 in promoting mammary tumorgenesis and metastasis. Increased expression of LCN2 is stimulated by the activation of the PI3K/AKT/NF-κB pathway in the tumour cells. Increased secretion of LCN2 from the tumour cells might directly affect MMP-9 activity to promote cell motility, the ER-independent growth or the transition to a more mesenchymal/aggressive phenotype. Alternatively, the secreted iron-deficient LCN2 may enter the normal cells through LCN2 receptor and sequester the intracellular iron causing cell death.

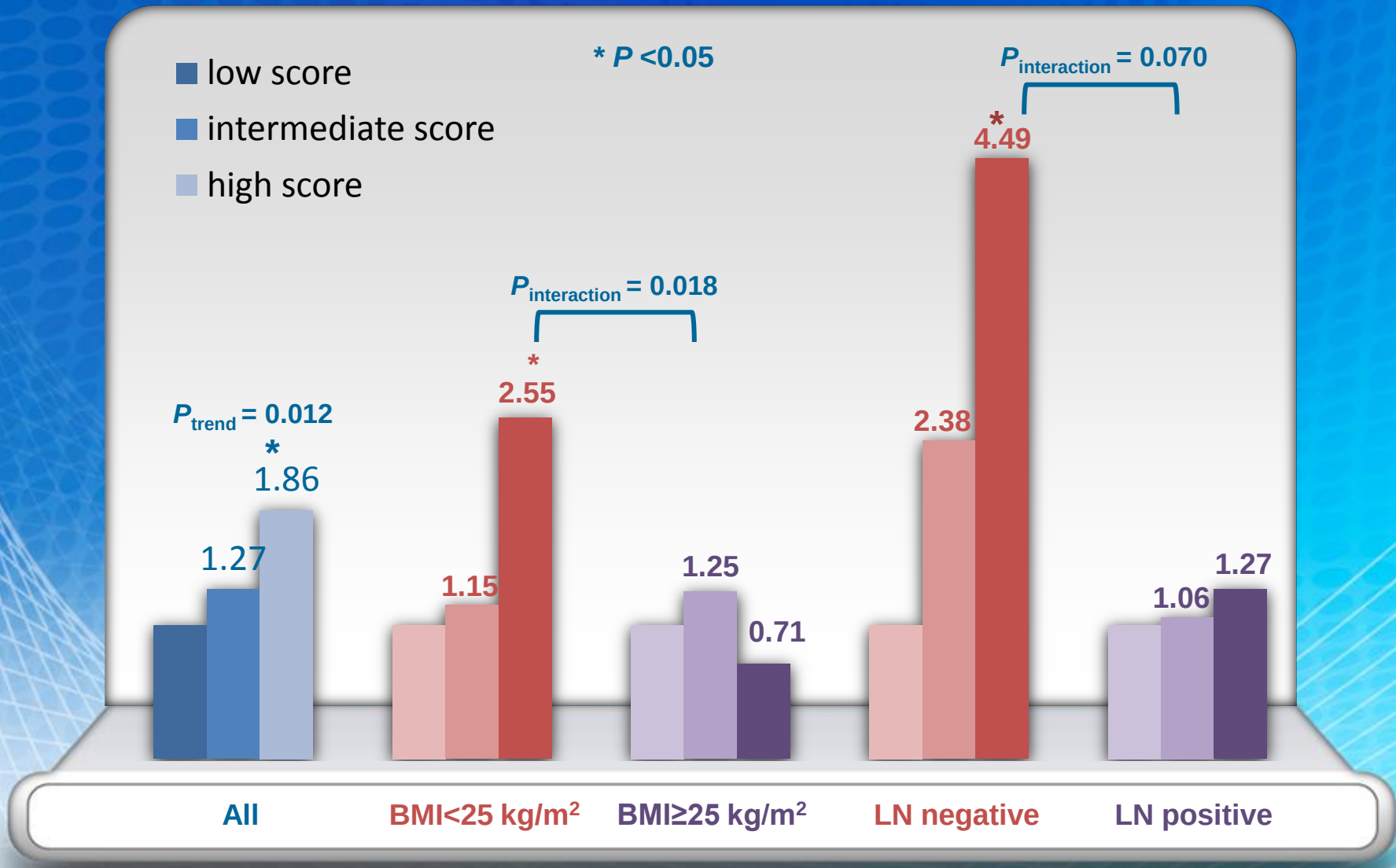
Leng et al, J Cell Physiol. 2011

Lipocalin-2 and MMP-9 for Breast Cancer Prognosis in SeBCS

		No. of All Patients	No. of Events	Adjusted HR	95% CI	*P*
Lipocalin-2 (ng/ml)	Continuous	303	87	1.05	1.02-1.08	0.003
	T1	100	22	1.00		
	T2	102	35	1.97	1.14-3.41	0.016
	T3	101	30	1.80	1.01-3.22	0.047
	P_{trend}					0.050
MMP-9 (ng/ml)	Continuous	303	87	1.04	1.00-1.06	0.104
	T1	100	22	1.00		
	T2	102	34	2.07	1.20-3.58	0.009
	T3	101	31	1.80	1.03-3.15	0.039
	P_{trend}					0.037
Combined	Continuous (0-4)	303	87	1.23	1.05-1.46	0.013
	Low (0-1)	109	29	1.00		
	Medium (2)	73	14	1.27	0.66-2.46	0.470
	High (3-4)	121	44	1.86	1.15-3.01	0.011
	P_{trend}					0.012

Sung and Kang et al, submitted

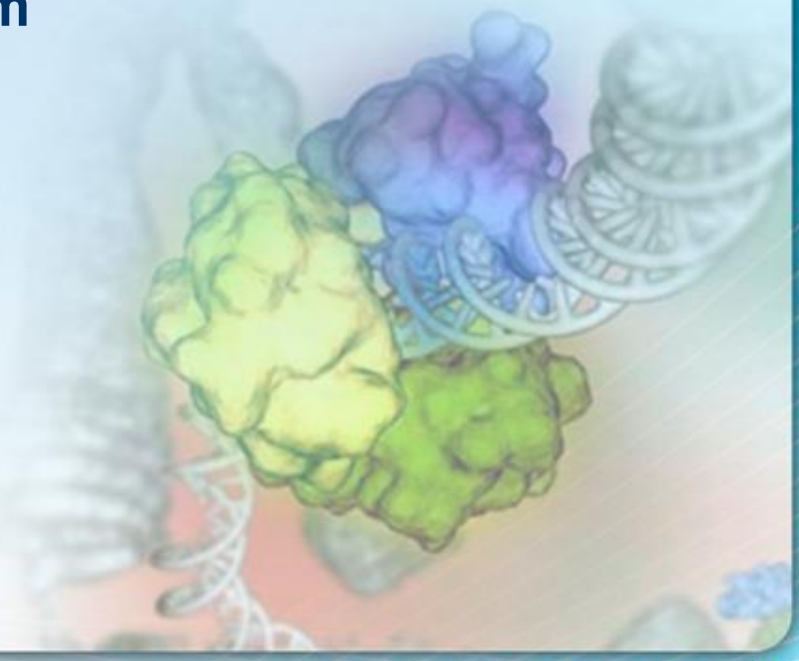
Association between Serum Lipocalin-2 & MMP9 levels and DFS by BMI and LN status



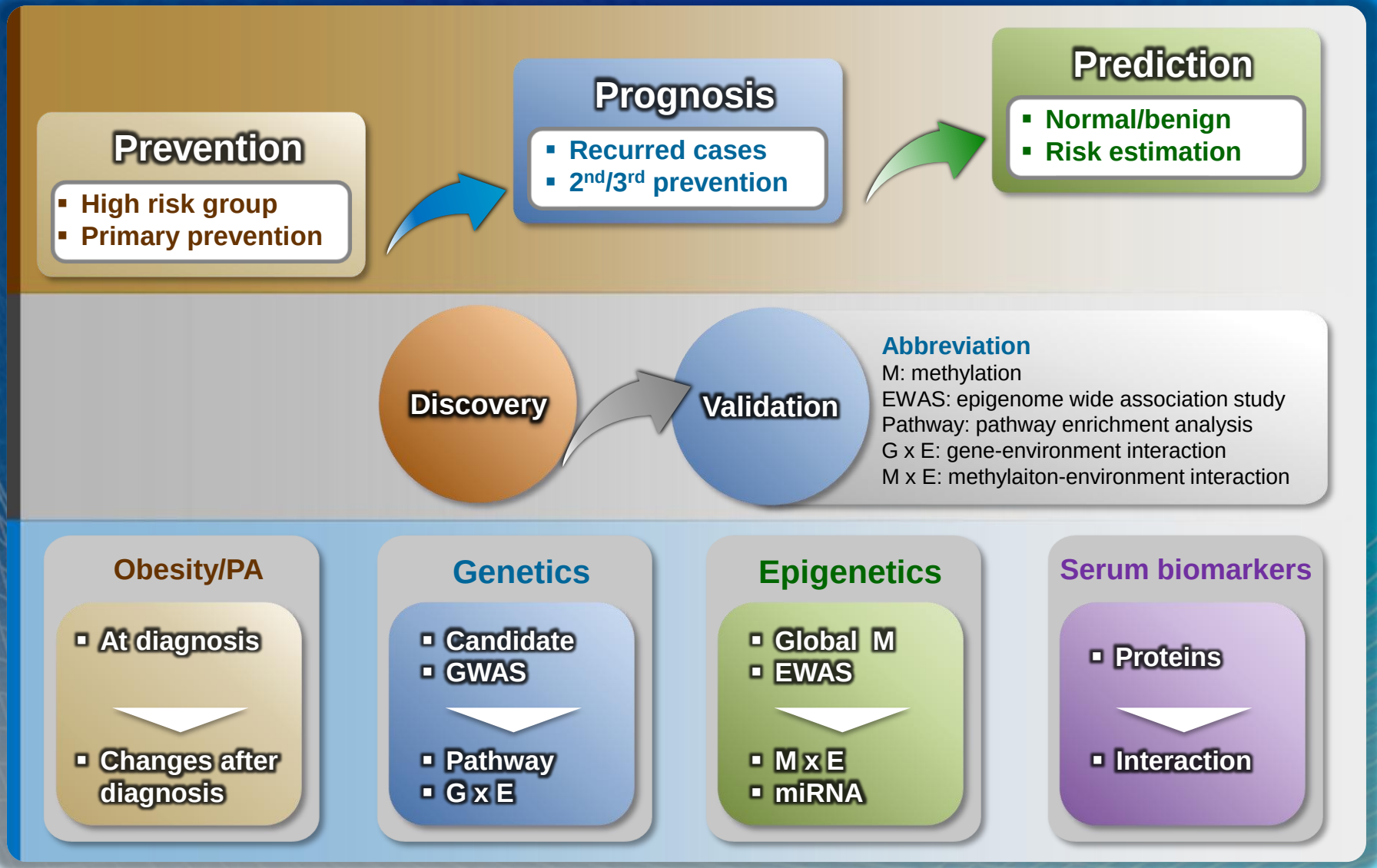
Sung and Kang et al, submitted MGEL

Summary

- Distribution and etiology of breast cancer subtypes are different across different populations.
- Obesity and physical activity might be independent factors of breast cancer survival but need more studies in Asian population.
- GWAS, methylation, and serum protein markers would be potentially useful biomarkers of breast cancer prognosis.



Current and Future Direction of SeBCS



Thank you

for attention